

Genetic Assay to Determine Genetic Relationship of Small Tributaries between the Tucannon River and Asotin Creek to Maintain the Tucannon River Steelhead Fishery

2016 Progress Report

Executive Summary

The Interior Columbia Basin Technical Review Team (ICTRT 2005) convened by National Marine Fisheries Service (NMFS), and the Snake River Salmon Recovery Plan (SRSRP 2006 and 2011) included natural origin summer steelhead (*Oncorhynchus mykiss*) in several small tributaries of the Snake River as part of the Tucannon or Asotin populations based on geographic location (Figure 1). The Washington Department of Fish and Wildlife (WDFW) collected tissues samples from these small Snake River tributaries. This project performed a genetic analysis from these tissue samples to determine genetic relationships of these small tributaries using genetic markers selected for their ability to distinguish Tucannon River steelhead from Asotin Creek steelhead (Seamons et al. 2015). To date, the panel of genetic markers has been finalized and genotyping of the tissue samples by the genetics lab is underway. Additional analysis is needed before a final report can be completed in the spring, 2017.

Experimental overview

WDFW will develop a high-throughput single-nucleotide polymorphism (SNP) panel based on analysis completed by Seamons et al. (2015) for collections from small Snake River tributaries (Table 1). WDFW will genotype a maximum of 50 individuals from each tributary for each year where we have samples. Sub-sampled fish from certain collections (where there is more than 50 samples) will represent the population return for that year based on known ages from scale samples collected. In total, 665 samples will be genotyped (Table 1).

Methods

A southeast Washington (SEWA) SNP panel was created by using restriction-site associated DNA methods (RAD-seq) to identify SNPs that differentiated Tucannon River, Asotin Creek, Touchet River, and Lyon's Ferry steelhead populations (Seamons et al. 2015). Seamons et al. (2015) selected 199 SNPs by examining significant global F_{ST} values between those four populations. Recent re-analysis of the RAD-seq data highlighted an additional 154 SNPs that had large minor allele frequency differences specifically between Tucannon River and Asotin Creek steelhead. A combination of the initial 199 SNPs (Seamons et al. 2015) and supplemental 154 SNPs were subsequently filtered for primer design. Of those, 239 SNPs were ordered as primer pairs for further evaluation and optimization. After two rounds of optimization genotyping, 54 SNPs were eliminated because of primer pair interactions or formation of primer dimers.

The final SEWA panel consists of 185 SNPs with two additional SNP loci, a sex determination SNP and species ID SNP (*O. mykiss*/*O. clarki*), for a total of 187. The samples identified in Table 1 were be genotyped with the SEWA panel using a cost effective method based on custom amplicon sequencing called Genotyping in Thousands (GTseq) (Campbell et al. 2015). GTseq is an efficient genotyping method that indexes individuals and then amplifies pools of targeted

SNPs. The pools are then sequenced, de-multiplexed, and genotyped by generating a ratio of allele counts for each individual.

This project will provide insight on the statistical measures of genetic relationship and summer steelhead population assignment to the Tucannon River and/or Asotin Creek from these small Snake River tributaries by using the SEWA GTseq panel. In addition, further insight and perhaps broader relevance may be discovered upon genotyping and analyzing all samples at the 269 SNP statewide standard steelhead GTseq panel.

Table 1. Steelhead tissue collections genotyped for assessing genetic relationships between Tucannon River, Asotin Creek, and nearby small Snake River tributaries.

Stream	Year	Sample Size	WDFW code
Almota	2011	50	11AF
Almota	2012	50	12BE
Alpowa	2008	50	08DK
Alpowa	2010	50	10CT
Alpowa	2011	50	11BH
Alpowa	2012	50	12DG
Alpowa	2013	50	13HB
Alpowa	2014	48	14IL
Alpowa	2015	50	15JZ
Asotin	2014	47	14AZ
Deadman	2012	10	12BC
Deadman	2013	7	13CL
Penewawa	2013	26	13CO
Penewawa	2014	32	14DI
Penewawa	2015	47	15DN
Tucannon	2014	48	14FG
Total		665	

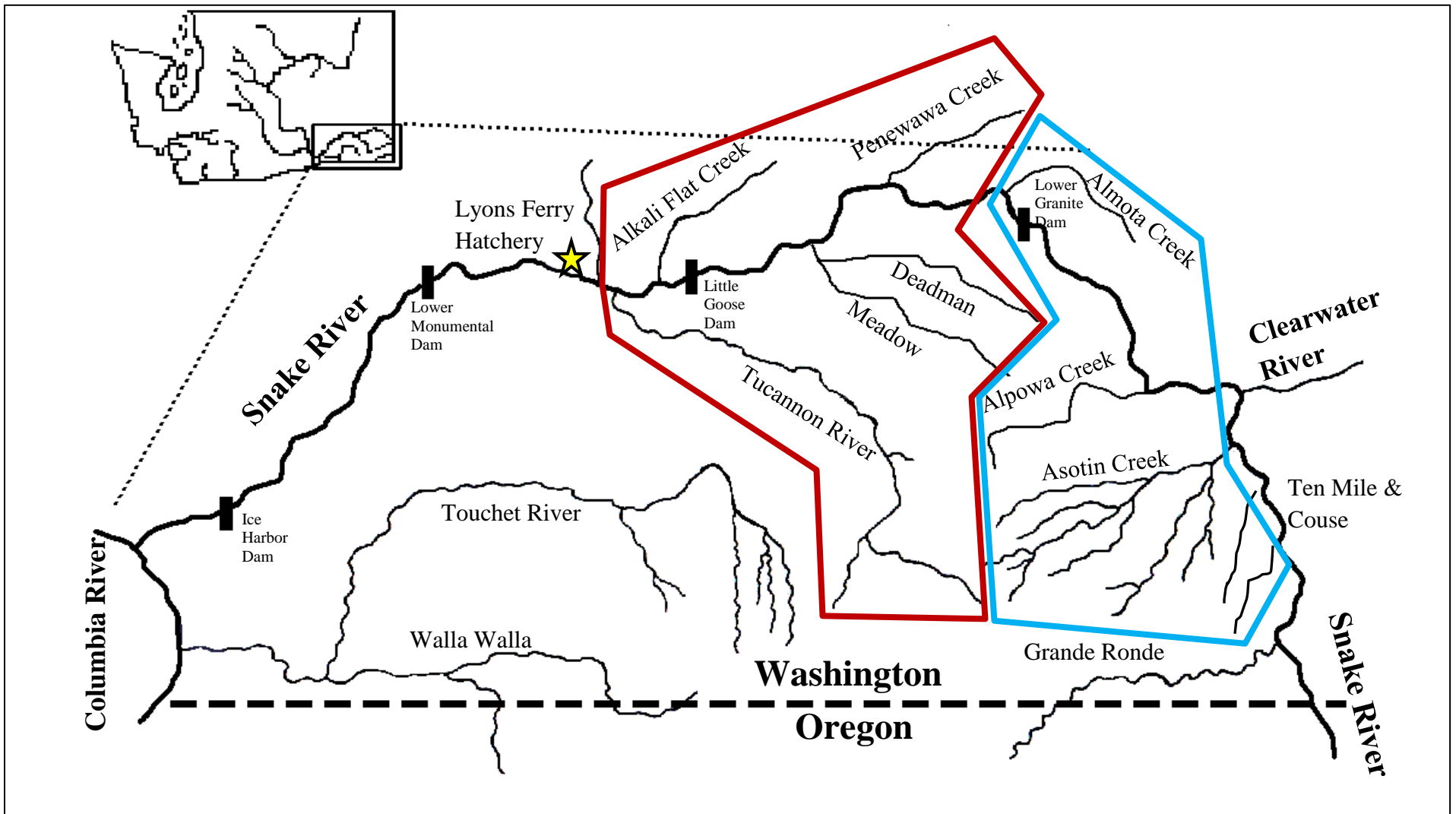


Figure 1. Map of southeast Washington showing the rivers containing managed summer steelhead populations. The red line surrounds the currently defined Tucannon Summer Steelhead population. The blue line surrounds the currently defined Asotin Creek summer steelhead population (adapted from Bumgarner and Dedloff 2015).

Budget Summary

Category	Cost (623 samples)	Funding Spent to Date
Development of high-throughput panel (consumables, goods and services)	\$6,200	\$6,200
Genotyping by sequencing (laboratory, consumables)	\$10,000	\$10,000
Labor (bioinformatics, analysis, and write-up)	\$17,000	\$17,000
Sub-total Direct	\$33,200	\$33,200
TOTAL	\$33,200	\$33,200

Request for 2016 is shown, but some of the planning and reporting work is provided as cost share using other WDFW funding. To date, all requested funds have been spent, but additional analysis and a final report will be completed with WDFW funding.

Results

The genetic analysis for this study is still underway, but should be completed in the spring of 2017. The genotyping of all individuals listed in Table 1 is in progress and analysis will immediately follow.

Benefits of Proposed Activity

The proposed project is critical for determining the potential contribution of small Snake River tributaries to the Tucannon population so the Tucannon Fishery may remain open, and also for meeting Endangered Species Act (ESA) recovery goals.

Maintenance of the Tucannon River steelhead fishery is very important to the angling public, local landowners to show that local recreational opportunities and economic benefits can be maintained while striving to achieve ESA recovery goals. The public can become frustrated and disinterested in the long-term recovery efforts unless they feel that local benefits can be maintained or enhanced. Some landowners and citizens have expressed much less interest in continuing efforts to enhance either stream habitat or to allow fish and habitat monitoring on private lands if the Tucannon River fishery might not be allowed to continue. The potential closure of this fishery has become quite heated for some of the public within the past few years and it has created political and social turmoil.

The Tucannon steelhead fishery is important to many landowners along the Tucannon River, as well as to businesses in the area (e.g. bait and tackle shop, RV Park, etc.), and to many anglers from a wide geographic distribution (Pullman, Walla Walla, Clarkston, Dayton, Spokane, etc.)

that have participated in this fishery, some for many years. The Tucannon fishery is very socially, culturally and economically important to citizens in southeast Washington.

The economic value of the Tucannon River steelhead fishery can be estimated based on the number of angler days expended per year. Estimate angler days/year has ranged from one to six thousand per year, with an average of 3,000 angler days annually. By applying the statewide standard of \$58 per angler day, the economic benefits of this fishery would range from \$58,000 - \$348,000 per year, with an average of ~\$174,000 per year.

Summary/Conclusion

The genetic analysis for this study is still underway, but WDFW will complete a final report in the spring of 2017. Following results, fishery managers in the area, along with NMFS, will decide how best to proceed with the steelhead population structure, and how these population will be accounted for and maintained in the future.

Any Outstanding Issues

The identification and optimization of the large panel of SNPs took longer than expected. The panel is now complete and genotyping is ongoing. Some discrepancies between the GTseq genotypes and the RAD-seq genotypes of individuals in the ascertainment panel (from Seamons et al. 2015) were found. This issue needs to be resolved prior to taking on similar projects in the future, but does not affect the outcome of this project.

Recommendations for future

In order to determine whether the Tucannon steelhead population is meeting the critical abundance threshold, as set by NMFS, we must be able to provide better estimates of total adult abundance or escapement to the Tucannon summer steelhead population. A critical piece of that information is determining which of these small tributaries along the Snake River can be included in the total Tucannon natural origin steelhead abundance estimate. This project is critical for that determination.

Unless we can show that the Tucannon natural origin steelhead population exceeds the critical threshold (285 adults) that is set in the Fisheries Management and Evaluation Plan (WDFW 2007), the Tucannon River steelhead fishery is in jeopardy of further restrictions or closures, regardless of how many adipose clipped hatchery fish may be available for harvest within the river.

This genetic study is part of a larger effort WDFW is conducting to better understand the interrelationships and adult abundances of the Asotin and Tucannon steelhead populations within 1) the Lower Snake River Compensation Plan Program, 2) Bonneville Power Administration funded projects that monitor summer steelhead returns in Asotin Creek and these small Snake River tributaries, and 3) WDFW tagging and smolt monitoring efforts for both hatchery and natural origin steelhead in the Tucannon River. Results from this project, in addition to the above projects will all be put together for a final WDFW management decision.

Literature Cited

- Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources* 15(4):855-867.
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- Seamons, T. R., Whitcomb, A., Mendel, G., Bumgarner, J.D., and K. I. Warheit. 2015. Development of a genetic assay to differentiate Tucannon River and Asotin Creek steelhead populations using RAD-seq methods. Final Report to the Columbia River Salmon and Steelhead Endorsement Board. 15 pages.
- WDFW. 2007. Fisheries Management and Evaluation Plan for the Incidental Take of Listed Species Submitted Under ESA Section 10/4(d). WDFW Recreation Fisheries That May Impact: Snake River Basin Steelhead DPS, Snake River Spring/Summer Chinook ESU, Snake River Fall Chinook ESU, and Snake River Sockeye ESU. September 19, 2007. 92 pages.